Rationale and design of the intervention in PHOspholamban RElated CArdiomyopathy STudy (i-PHORECAST)

CHAPTER 11

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Abstract

Background

The p.Arg14del (c.40_42delAGA) phospholamban (PLN) mutation is a Dutch founder mutation causing dilated cardiomyopathy (DCM) and/or arrhythmogenic cardiomyopathy (ACM). It is associated with an increased risk of malignant ventricular arrhythmias and heart failure, which has been ascribed to cardiac fibrosis. Importantly, cardiac fibrosis appears to be an early feature of the disease, occurring in many presymptomatic mutation carriers before onset of overt disease. As with most monogenetic cardiomyopathies, no proven treatment is available for presymptomatic mutation carriers.

Aims

PHORECAST (the intervention in PHOspholamban RElated CArdiomyopathy STudy) is designed to demonstrate that pre-emptive treatment of presymptomatic PLN p.Arg14del-carriers using eplerenone, a minerocorticoid receptor antagonist with established antifibrotic effects, can reduce disease progression and postpones the onset of overt disease.

Methods

iPHORECAST has a multicenter, prospective, randomized, open-label, blinded endpoint (PROBE) design set-up. A total of 150 presymptomatic PLN p.Arg14del mutation carriers (n=75 per group) will be randomized to either eplerenone 50 mg once daily or no treatment. The primary endpoint is disease progression, defined as a composite endpoint of cardiac magnetic resonance parameters (CMR; LV and RV volumes, systolic function and fibrosis), electrocardiographical parameters (QRS voltage; ventricular ectopy), signs and/or symptoms related to DCM and ACM, and cardiovascular death. The follow-up period is a minimum of 3 years.

Conclusion

iPHORECAST will address if pre-emptive treatment of PLN p.Arg14del mutation carriers with eplerenone can prevent or delay disease onset. It is the first prospective randomized trial to address the efficacy of preventive treatment in presymptomatic carriers of a known pathogenic-cardiomyopathy-associated mutation.

Introduction

DCM and ARVC are both clinically heterogeneous diseases of the myocardium, associated with mechanical and/or electrical dysfunction.[1,2] Clinically, ARVC is characterized by right ventricular (RV) arrhythmias and sudden cardiac death, often preceding structural changes.[3,4] DCM, on the other hand, is characterized by left ventricular (LV) contractile dysfunction and progressive heart failure, with arrhythmias often present but less prominent.[5] In the Netherlands, 15% of idiopathic dilated cardiomyopathy (DCM) patients and 12% of arrhythmogenic right ventricular cardiomyopathy (ARVC) patients carry a single mutation in the gene encoding phospholamban, p.Arg14del (c.40_42delAGA).[6] Haplotype analysis has revealed this mutation to be a common founder mutation7 and more than 1000 mutation carriers have been identified in the Netherlands to date. Therefore, it is currently the single most prevalent cardiomyopathy-related mutation identified in the Netherlands, with an estimated prevalence in large parts of the country of 1:1000. Moreover, the mutation has also been identified in a number of European countries (Germany, Greece, Belgium, Norway, the UK and Spain), as well as in Canada and the USA, attesting to its global significance. To promote research and improve the quality of diagnostics and therapy in carriers of the PLN p.Arg14del mutation, we have set up a registry called PHORECAST (PHOspholamban RElated CArdiomyopathy Study; http://www.phorecast.nl).

The cardiac phenotype of PLN p.Arg14del mutation carriers is typically characterized by the presence of malignant ventricular arrhythmias, sudden cardiac death and heart failure.[6,8] An experimental murine overexpression model of this PLN mutation found this cardiomyopathy to be characterized by severe cardiac fibrosis and progressive myocardial loss and dysfunction.[9] Importantly, in our patients, we observed LV myocardial fibrosis even in the presence of preserved (>45%) LV ejection fraction (LVEF). This suggests that extensive remodeling occurs early on in PLN p.Arg14del mutation carriers, even before the onset of overt disease, and is reflected by a high prevalence of low voltage criteria and negative T waves on the surface electrocardiogram (ECG) in these presymptomatic subjects.[6,10,11] It is probable that this myocardial fibrosis is at least partly responsible for the subsequent development of electrical instability and LV impairment. In general, previous studies suggest that cardiac fibrosis is an extra independent risk factor in DCM patients.[12-16] An earlier study by our group showed that an LVEF of less than 45% (rather than 35%) is an independent risk factor for ventricular arrhythmias (VA).8 We refined this finding in a more recent study that showed LV-LGE on CMR is an even stronger risk factor than LVEF. In fact, even in the setting of preserved LVEF, the mere presence of LV-LGE is associated with a higher risk of VA in PLN p.Arg14del mutation carriers.[11]

Analogous to other inherited cardiomyopathies, the natural course of PLN p.Arg14del cardiomyopathy is age-related (age-related penetrance). After a presymptomatic phase of variable length many PLN p.Arg14del-carriers progress to overt disease characterized by high rates of malignant ventricular arrhythmias and end-stage heart failure, and this often necessitates internal cardioverter defibrillator (ICD) therapy and heart transplantation.[6] When there is overt DCM and/or arrhythmogenic cardiomyopathy (ACM), treatment is initiated in these patients according to current guidelines and cohort-specific insights (e.g. ICD in mutation carriers with an EF <45%). [8]

Using family cascade family screening, a large subgroup of presymptomatic family members carrying the PLN p.Arg14del mutation have been identified, providing a unique window of opportunity to preventively treat these mutation carriers before symptoms occur. However, no evidence-based treatment is available for these presymptomatic mutation carriers even though early treatment, before the onset of any symptoms, is potentially of major prognostic importance. Early therapeutic intervention might prevent sudden cardiac death, disease development and progression.

Eplerenone is, due to its drug class- and specific characteristics, particularly interesting as an early treatment for PLN p.Arg14del mutation carriers. It is a selective mineralocorticoid-receptor blocking agent with potent antifibrotic properties,[17-23] and it is usually very well tolerated with minimal side-effects. Mineralocorticoid-receptor blocking agents are recommended for symptomatic patients with heart failure (NYHA class 2-4) with reduced LV ejection fraction despite optimal treatment with an ACE-inhibitor, a beta-blocker and diuretics (class IA-indication).[24] The goal of iPHORECAST is to address if pre-emptive treatment with eplerenone in PLN p.Arg14del mutation carriers will prevent or delay disease onset. The study will provide evidence as to whether it is efficacious to administer preventive treatment in presymptomatic carriers of a known pathogenic cardiomyopathy-associated mutation, comparable to a previous non-randomized trial with diltiazem in hypertrophic cardiomyopathy.[25]

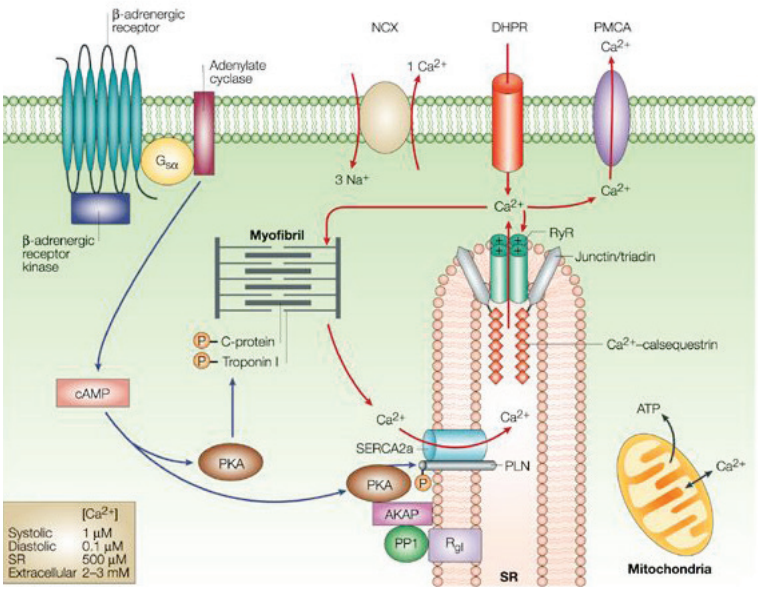

Figure 1. Function of Phospholamban (PLN). *PLN is a reversibly phosphorylated transmembrane protein that binds to and regulates the activity of SERCA2a, the sarcoplasmic reticulum Ca2+-ATPase (SERCA2a) pump. (From: MacLennan et al. Nat Rev Mol Cell Biol. 2003)*

Study Design

Objectives

The objective of this trial is to show that eplerenone treatment can reduce progression of disease in presymptomatic PLN p.Arg14del mutation carriers.

Study population

Specific inclusion and exclusion criteria are listed in *Table 1*. In the Netherlands, all known PLN p.Arg14del mutation carriers are registered in the PHORECAST registry (PHOspholamban RElated CArdiomyopathy Study; **http://www.phorecast.nl**), including all presymptomatic carriers. We use the PHORECAST registry to recruit eligible presymptomatic subjects. Study participants must be between 18 and 65 years of age and have a New York Heart Association (NYHA) functional class of 1 and an LV ejection fraction of more than 45% (measured by CMR). For ARVC and DCM criteria we use, respectively, the revised task force criteria[26] and Mestroni criteria.[27]

Table 1. Key inclusion- and exclusion criteria

Key inclusion criteria
• PLN p.Arg14del mutation carriers
• Age ≥18 and ≤ 65 years
• New York Heart Association functional class ≤ 1
Key exclusion criteria
• Palpitations necessitating treatment (at the discretion of the attending physician)
• A diagnosis of DCM (according to the Mestroni criteria[27]). Note: regional LV wall motions abnormalities are acceptable.
• A diagnosis of ARVC (according to the task force criteria[26])
• Global or regional RV dysfunction and/or structural alterations (according to task force criterion 1[26])
• Ventricular premature complexes >1000 during 24hours Holter-monitoring
• Non-sustained ventricular tachycardia during Holter-monitoring or exercise-testing
• History of sustained ventricular tachycardia or ventricular fibrillation
• Hypertension requiring the use of antihypertensive drugs, or when this is anticipated within the coming 3 years
• Evidence of ischemic heart disease
• Treatment with cardioactive medication
• Hyperkalemia (serum potassium >5.0 mmol/l)
• Severe renal dysfunction (eGFR <30 ml/min/1.73 m^2)
• Severe hepatic impairment (Child-Pugh class C)
• Women who are currently pregnant or report a recent pregnancy (last 60 days) or plan on becoming pregnant.
• Concomitant use of CYP3A4-inhibitors
• Concomitant use of NSAIDs
• Concomitant use of potassium-sparing agents
• Known intolerance or contraindication for aldosterone antagonists
• Participation in another drug trial in which the last dose of drug was within the past 30 days.
• Contra-indications for CMR (claustrophobia, metal devices)
• Subjects unable or unwilling to provide written informed consent

Note: presence of late gadolinium enhancement on CMR is not an exclusion criterion

Design (*figure 2*)

This outpatient study is a multicenter, prospective, randomized trial with blinded assessment of endpoints design (PROBE).[28] The participating centers are the University Medical Center Groningen (which is the coordinating center and CMR core lab); Amsterdam University Medical Center; Antonius hospital, Sneek; and University Medical Center Utrecht. Mutation carriers will be randomly assigned to one of the two arms of the study: either eplerenone or control (no treatment) in a ratio of 1:1. Mutation carriers taking other cardiovascular medication, including diuretics, ACE-inhibitors, angiotensin receptor blockers and mineralocorticoidreceptor antagonists, beta-blockers, and anti-arrhythmic medication, are excluded from this trial.

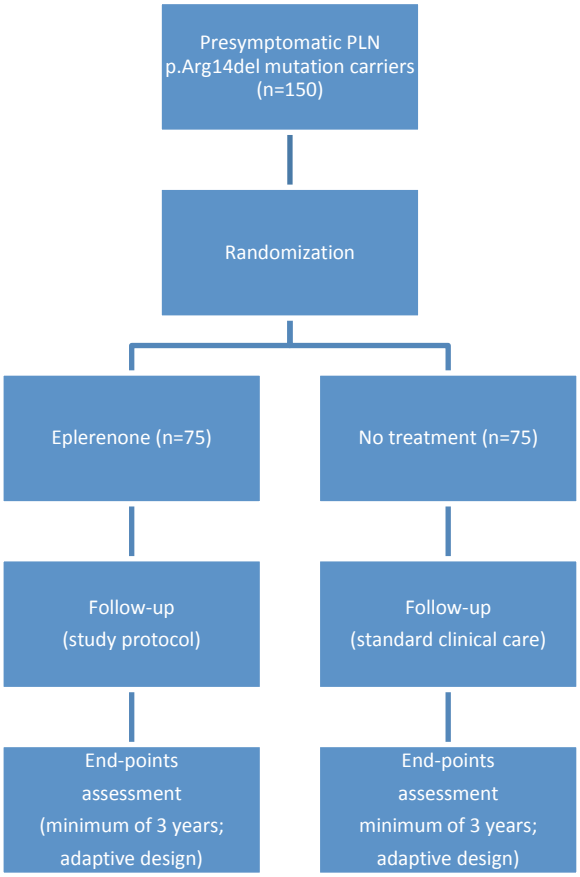

Figure 2. Study design

Ethical considerations

This study will be conducted in accordance with the principles stated in the revised Declaration of Helsinki (Seoul 2008) and in accordance with the Medical Research Involving Human Subjects Act. The Ethics Committee of the University Medical Center Groningen, the Netherlands, approved this study (METc 2013-272; ABR NL43771.042.13; Eudra-CT 2013-001067-23), and the study protocol was also submitted to the ethics committees of each participating hospital. Written informed consent must be obtained from every subject before entry into the study. An independent data and safety monitoring board (DSMB) will be installed from the start of the study to safeguard the interests of i- PHORECAST trial participants, assess the safety of the interventions during the trial, and monitor the overall conduct of the clinical trial. The study has been registered and will be kept up-to-date in the clinicaltrials.gov-register under number: NCT01857856.

Treatment

In the treatment arm, eplerenone will be initiated at 50 mg orally once daily, starting the day of randomization. Mutation carriers will be maintained at this target dose or the maximum tolerated dose until the end of the study. Serum potassium and eGRF will be monitored during the follow-up period, 1-2 week(s) after start and yearly, and will be documented together with the other investigations and vital parameters. The dose will be reduced if needed, i.e. in case of hyperkaliemia (serum potassium >5.5 mmol/l). The dose of 50 mg once daily is the recommended dose in patients with heart failure. Mutation carriers will be followed up in the cardiology outpatient clinic at each center yearly according to routine clinical care. Physical examination, blood sampling, electrocardiogram, Holter monitoring, and ergometry will be performed every 12 months. CMR imaging and SA-ECG will be performed at the start and end (36 months) of the study (*table 2*).

Table 2. Overview of study procedures

	Visit 1 start	Visit 2 1 week	Visit 3 1 year	Visit 4 2 years	Visit 5 3 years
Visit to outpatient clinic	✔		✔	✔	✔
History and current status	✔		✔	✔	✔
Physical examination	✔		✔	✔	✔
Electrocardiogram	✔		✔	✔	✔
Echocardiogram	✔		✔	✔	✔
Holter monitoring	✔		✔	✔	✔
Ergometry	✔		✔	✔	✔
Signal-averaged ECG	✔				✔
CMR	✔				✔
Blood analysis	✔	✔*	✔*	✔*	✔

**only for subjects randomized to eplerenone*

Note: Subjects undergo several investigations as summarized in the above schedule. They are requested to visit the outpatient clinic at baseline and at one year-intervals thereafter. Subjects randomized to eplerenone visit the outpatient clinic or their GP after 1 week to check serum potassium and eGRF, and this will also be checked in these subjects at the yearly visits. Of note, all investigations except for blood analysis are already performed as part of routine clinical care, which routinely includes evaluation of symptoms and signs of DCM and ARVC. For the purpose of the study we will collect the available data and use these investigations.

Study endpoints

The primary endpoint is disease progression defined as a composite endpoint (*table 3*). If at least one of the components is reached, the primary endpoint is reached. *Table 3* also depicts the secondary endpoints of the study. Outcomes will be assessed by an independent endpoint committee blinded to the allocated group. After a minimum follow-up of 3 years, endpoints will be assessed by an independent endpoint adjudication committee. In case of a favorable trend (but no significant effect as yet) the DSMB may advise continuing the study for 2 more years ("adaptive design").

Table 3. Primary- and secondary endpoints

Primary Endpoint*

- LV end-diastolic volume, increase >10% (assessed with CMR)
- LV ejection fraction, absolute decrease >5% (assessed with CMR)
- RV end-diastolic volume, increase >10% (assessed with CMR)
- RV ejection fraction, absolute decrease >5% (assessed with CMR)
- Late enhancement, absolute increase >5% (assessed with CMR)
- QRS voltage, decrease >25% (ECG, measured in I, II and III in mV)
- Occurrence of non-sustained ventricular tachycardia (Holter monitoring, exercise testing)
- Heart failure symptoms and/or signs or arrhythmias necessitating medical treatment according to the guidelines and likely due to arrhythmogenic cardiomyopathy

Secondary Endpoints

- All individual components of the primary endpoint
- Diagnosis of DCM (according to Mestroni criteria[27])
- Diagnosis of ARVC (according to revised task force criteria[26])
- Development of global or regional dysfunction and structural alterations, (according to revised task force criterion 1[26])
- QRS-axis (12-lead ECG)
- Conduction intervals (PR-interval, QRS-duration; (12-lead ECG; SA-ECG))
- STT-segment changes (12-lead ECG)
- Change in pre-specified biomarkers (see table 4)
- Occurrence of sustained ventricular tachycardia or ventricular fibrillation
- Hospitalization for a cardiovascular reason

**The primary endpoint is defined as a composite endpoint. If at least one of the components is reached, the primary endpoint is reached.*

Sample size estimation

This clinical trial was designed based on the assumptions that 50% of presymptomatic PLN p.Arg14del mutation carrier group without treatment will reach the primary endpoint within 3 years and that treatment with eplerenone is expected to reduce this percentage by 50%. To ensure at least 80% power to detect a difference between the control group and the eplerenone group at a significance level of 0.05, a total of 128 mutation carriers (64 in each group) are needed. To account for 10-15% loss to follow-up during the study, we aim to include 150 subjects in this study.

Randomization and blinding

Central randomization will be conducted by the Trial Coordination Center of the University Medical Center Groningen (**www.tcc.umcg.nl**). Randomization will be stratified according to participating centers to ensure a similar sample distribution between the two test groups. According to the PROBE design, randomization will be blinded to the CMR core lab in the UMCG that will assess the CMR images. An independent endpoint adjudication committee will assess the other components of the composite endpoint.

Statistical analysis

All eligible mutation carriers randomized into the study who have received at least 1 dose of eplerenone will be used in the analyses of the primary and secondary efficacy endpoints as well as in the safety analysis. Safety will be assessed by summarizing the incidence and type of adverse events and the changes in laboratory parameters. The proportion of mutation carriers experiencing serious adverse events and the proportion of mutation carriers with noteworthy changes in laboratory parameters will be compared between treatment groups. The analysis of primary endpoints and secondary endpoints will be performed according to their assigned treatment group in accordance with the intention to treat principle. Additional analysis sets may be used in exploratory analyses. The number of subjects withdrawing from the study will be tabulated by their reasons for withdrawal and by treatment group. Potential biases due to withdrawal of subjects will be investigated. Baseline characteristics will be compared using Chi-square test (or Fisher Exact test if appropriate). If differences are found, caution will be taken when interpreting the results of the analyses between groups, and methods may be modified to adjust for this difference.

The primary outcome will be presented by Kaplan-Meier curves for the treatment group, followed by the stratified log-rank test using stratification variables. Multivariate analysis will also be used to compare the endpoints between the two groups adjusting for other variables including sex, age and screening centers. For missing data, the last observations will be used. Unless stated otherwise, all secondary analyses will be performed using the same subjects included in the primary analysis. Secondary variables will be analyzed using an appropriate statistical test, depending on the nature of the variable. Changes in parameters over time in the different treatment groups will be analyzed using repeated measurement analysis or techniques that evaluate the timing of endpoints, when appropriate. For all tests, a p-value <0.05 is considered statistically significant. Statistical analysis will be performed in SPSS (IBM SPSS Inc., Chicago, IL, USA; newest version at moment of analysis).

Study organization and monitoring

This is an investigator-initiated study organized by the executive steering committee, which is part of the department of Cardiology, University Medical Center Groningen, Groningen, the Netherlands. The participating medical centers and the principal investigators at each center are listed in Appendix I. The executive steering committee is responsible for overall supervision of the study, policy decisions, protocol amendments, publications and presentations. An independent DSMB will monitor the overall conduct of this study by receiving and reviewing reports of serious adverse events, reviewing periodic reports of safety data, and establishing 'stopping rules' for the trial for safety reasons only.

Biomarkers

Venous blood samples from all participants will be stored and analyzed for levels of biomarkers of fibrosis[29] as well as for biomarkers of apoptosis, hemodynamic status and inflammation (*table 4*; including manufacturer, sample size and tube type). We will investigate whether these biomarkers predict disease progression. In addition to the volume of blood needed to measure these biomarkers, additional blood (serum and plasma) will be taken and stored to allow additional analyses in the future (samples stored for a maximum of 15 years). The amount of blood taken per venipuncture is 20 ml (at the start of the study and after three years). Patients will be asked to give their written informed consent for these additional analyses at the start of the study.

Table 4. Biomarkers

Biomarker (abbreviation)	Kit manufacturer	Sample size (per sample)	Tube type
Procollagen type I amino-terminal peptide (PINP)	Orion Diagnostica, Finland (RIA)	50 µl	Serum
Procollagen type III amino-terminal peptide (PIIINP)	Orion Diagnostica, Finland (RIA)	200 µl	Serum
Type 1 collagen telopeptide (ICTP)	Orion Diagnostica, Finland (RIA)	100 µl	Serum
Galectin 3	BG Medicine, USA (ELISA)	100 µl	EDTA
Osteopontin	IBL International, Germany (ELISA)	100 µl	EDTA
Osteonectin (SPARC)	Adipobiotech, USA (ELISA)	50 µl	EDTA
Periostin	USCN Life Science inc, USA	100 µl	EDTA
Aldosterone	BioVendor, Czech republic (ELISA)	200 µl	Serum
Renin (PRC)	Alpco immunoassays, USA (ELISA)	50 µl	Serum
Cortisol	BioVendor, Czech republic (ELISA)	100 µl	Serum
Kidney injury molecule-1 (KIM-1)	USCN Life, China (ELISA)	200 µl	EDTA
Neutrophil gelatinase-associated lipocalin (NGAL)	USCN Life, China (ELISA)	200 µl	EDTA
Cystatin-1	USCN Life Science, USA	100 µl	EDTA
NT proBNP	USCN Life Science, USA	100 µl	EDTA
Total (calculated for 2 samples)		3300 µl Serum: 1400 µl EDTA: 1900 µl	

Discussion

The Dutch p.Arg14del founder mutation in the gene encoding for phospholamban (PLN) causes DCM and ARVC and is associated with an increased risk of malignant ventricular arrhythmias and heart failure.[6,10,11] Although DCM and ARVC are considered separate entities by both the American Heart Association[30] and the European Society of Cardiology[31], they do have overlapping clinical features[32,33], supporting the concept of ACM.[4] This notably true in PLN p.Arg14del cardiomyopathy, where we find a significant clinical overlap between DCM and ARVC phenotypes in a substantial number of patients in whom left- or right-sided forms may predominate.[6] Because malignant ventricular arrhythmias can be the first presentation of this biventricular cardiomyopathy, early identification and, hopefully in the near future, preventive treatment of presymptomatic mutation carriers at risk is essential.

The present multicenter prospective randomized clinical trial aims to show that pre-emptive treatment (eplerenone) can prevent or delay disease onset in presymptomatic mutation (PLN p.Arg14del) carriers, comparable to a previous non-randomized trial with diltiazem in hypertrophic cardiomyopathy.[34] Given eplerenone' s potent antifibrotic effects, we anticipate it will have a strong beneficial effect in retarding disease progression. Eplerenone has been shown to be very effective and safe, even in patients with advanced cardiac disease (advanced heart failure). It is usually very well tolerated with minimal side-effects. The most significant side-effect is hyperkalemia, but this only occurs in clinical practice in combination with other potassium-sparing drugs and/or renal impairment. The presymptomatic subjects in the present study, who are unlikely to be using these medications, are therefore much less vulnerable and by using strict inclusion and exclusion criteria and follow-up (monitoring) we are convinced the use of eplerenone in the present study is very safe.

The primary objective of this study is to assess the effect of eplerenone on disease onset and progression. To improve statistical power, a composite endpoint of cardiac magnetic resonance parameters (CMR; LV and RV volumes, systolic function and fibrosis) and electrocardiographical parameters (QRS voltage; ventricular ectopy) and of signs and/or symptoms related to DCM and ACM and cardiovascular death will be adopted as the primary endpoint. Serum markers of fibrosis, apoptosis, hemodynamic status and inflammation will be measured as part of the secondary endpoint to investigate whether they can predict disease progression. These biomarkers will therefore also be used during follow-up to provide prognostic information.

In conclusion, PLN p.Arg14del cardiomyopathy is a malignant biventricular cardiomyopathy characterized by malignant ventricular arrhythmias and sudden cardiac death, sometimes at a young age, in a subset of mutation carriers. Early intervention is therefore essential, yet there is no evidence- based preventive treatment. State-of-the-art CMR measurements reflecting both cardiac structure, function and location and extent of fibrosis are the main primary endpoint. iPHORECAST is designed to examine whether pre-emptive treatment with eplerenone in asymptomatic PLN p.Arg14del mutation carriers can prevent or delay disease onset in a multicenter and randomized manner.

Adaption of the study design due to slow recruitment – April 1, 2017

The first participant was included in the study May 13, 2014. Because of slow recruitment, it was decided on April 1, 2017 to stop further inclusion of participants but to continue and complete the study with the included cohort (N=81). As a consequence, it is not realistic to assume that enough participants will reach the primary endpoint or that a significant difference will be demonstrated between the eplerenone group and the control group regarding the primary endpoint (*table 3*). The statistical analysis will be amended accordingly: instead of focusing on the composite dichotomous endpoint, i.e. using the pre-specified cut-off values, individual components of the primary endpoint will now be analyzed on a continuous scale and combined to look for significant trends. The decision to amend the design of the study (i.e. to stop inclusion of participants and to continue with the included cohort) was supported by the DSMB and medical ethics committee. The primary objective remains the same, i.e. to show that eplerenone retards disease progression.

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Disclosures

The authors report no conflict of interest.

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